Isolation and identification of restriction endonuclease BseAI

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BseA I, an isoschizomer of BspM II (1) has been purified from *Bacillus stearothermophilus* species.

Figure 1. BseA I digests; lane 2: SV40, 3: ϕ X 174, 4: pBR322 5: T7, 6: Adeno 2, 7: Lambda DNA, lanes 1 and 8 lambda Hind III size standard.

Figure 2

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REFERENCES

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2. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci USA 74, 5463-5467

BseA I recognises the sequence 5'TCCGGA3' and cleaves between T and C giving four 5' overhanging bases. The enzyme was purified using the following Chromatographic steps: 1) Heparin-Agarose, 2) Phosphocellulose, 3) ds DNA-cellulose. The enzyme was free of contaminating nuclease activity. After 100 fold overdigestion on lambda DNA greater than 95% of the DNA fragments can be ligated and greater than 95% can be recut by BseA I. Optimal conditions for BseA I activity are 150mM NaCl, 10mM Tris (pH 7.5), 10mM MgCl₂ at 65°C. The fragments produced by BseA I digestion of SV40, ϕ X 174, pBR 322, T 7, Adeno 2 and lambda DNA match those predicted by cleavage at the sequence TCCGGA (figure 1, lanes 2-7). A pUC 19 recombinant clone which contained a recognition site for the enzyme was digested by the enzyme BseA I then annealed with forward or reverse sequencing primers and extended with Klenow enzyme in the presence of α^{32} P-dATP. Dideoxy sequencing reactions were performed at this region with the same primers and run in parallel with the extended products (2). Results in figure 2 show that extended products of the forward (lane 1) or the reverse (lane 2) sequencing primers following cleavage with BseA I comigrate with the 3'G in the 5'...TCCGGA...3' sequence. Therefore BseA I recognises and cleaves the following sequence.